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A Study of Antimicrobial Activities of Plant Extracts (*Talisia squarrosa* and *Mora gonggrijpii*)

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The anti-microbial activity of extracts from different vegetative parts of two plant species found in Guyana was evaluated. The selected plants belong to two families: Sapindaceae: Talisia squarrosa; and Fabaceae: Mora gonggrijpii. The standard method was used to obtain plant extracts. The extracts were evaluated in independent bioassays against fungal and bacterial pathogens using different concentrations. The antimicrobial activities of Talisia squarrosa and Mora gonggrijpii were studied using the poison plate method, well diffusion method (antifungal activities) and streak plate method (antibacterial activities). Mora gonggrijpii showed 50% inhibition of fungus at concentration of 0.43g/ml. Phytochemical examination of Talisia squarrosa and Mora gonggrijpii revealed the presence of anthracenosides, tannins, anthocyanins, couramins, saponins and steroid glycosides. The activities displayed by the extracts can be attributed to the presence of these phytochemicals.

Key words: *Talisia squarrosa, Mora gonggrijpii,* antimicrobial activity, phytochemical analysis. Poison plate, Well plate diffusion.

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. There is yet much untapped ethnobotanical and ethnopharmacological information to be harnessed from our rich biodiversity. The isolation of biologically active natural products may be considered as a necessary tool in the sustained utilization of our vast biodiversity.

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The search for new bioactive compounds must be an on-going one since it is inevitable that efforts be sustained for the exploration of new efficient, safe and inexpensive anti-infective agents. Hostettmann *et al* (2000) reported that there is a growing trend in the misuse of antibiotics coupled with the resistance of previously sensitive microorganisms. One way to address this is to engage in research that will seek to develop new antimicrobial drugs. Research of this nature is very significant since it will add to the growing body of scientific knowledge in the Faculties of Natural Sciences, Agriculture and Forestry.

Secondary metabolites such as flavanoids (Tsuchiya *et al.,* 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al.,* 1988), terpenoids (Scortichini and Pia Rossi,

1991), sesquiterpenes (Goren, 1996) to name a few possess effective antimicrobial properties against a wide range of microorganisms.

Sapindaceae, a tropical and sub tropical family of 158 genera and 2230 species, is economically very important. This family is represented by five genera, Dodonaea; Sapindus, Cupaniopsis, Cardiospermum and Stocksia. The species of these genera are rich in biologically active components, e.g., saponins, flavonoids, steroids, proteins and lipids (Ahmad, 1992).

The Fabaceae are mostly herbs but also include shrubs and trees found in both temperate and tropical areas. They comprise one of the largest families of flowering plants, numbering some 400 genera and 10,000 species. The leaves are stipulate, nearly always alternate, and range from pinnately or palmately compound to simple (Carr, 2006). The species of these genera have produced a number of secondary metabolites such as tannins, saponins, anthracenosides, steroids and anthocyanins (Jackson *et al.*, 1995).

The plant families, Fabaceae and Sapindaceae, have a history of uses for the treatment of various ailments. Several investigations involving phytochemical and antimicrobial assays (*Lecaniodiscus cupanoides,* (Adesegun, 2003) *Dodonaea viscose,* (Khalil, 2006) *Gliricidia sepium* (Ganesan, 1994) have been conducted. The present study examines the antimicrobial activities of the alcoholic extracts of *Talisia squarrosa* and *Mora gonggrijpii.*

Methodology

Collection and Identification of plant materials

Plant materials were collected from sample plots at the Forestry Training Centre Inc., Mariwa, Essequibo; in June 2009. Botanical identification in the field was performed by Isaac Johnson, tree-spotter for the Forestry Training Centre Inc. The voucher specimens were deposited at the herbarium at the Centre for the Study of Biological Diversity, University of Guyana, Turkeyen Campus.

Extraction of plant materials

Talisia Squarrosa root was collected and shade dried, to avoid the denaturing in sunlight of any useful compound. The dried root was pulverized using a Thomas-Willey mill. An alcoholic extract was prepared by soaking 100g of the powdered root in 500ml of ethanol and left to stand for 24 hours. The crude extract was filtered using Whatmann 41 filter paper, and the residue was soaked in an additional 500ml of ethanol and allowed to stand for a further 24 hours. The crude extract was filtered using Whatmann 41 filter paper. The combined extracts were concentrated using a rotary evaporator at 40 ^{o}C, to yield a crude orange-brown solid. The crude extract was re-suspended in ethanol at a concentration of 0.43g/ml and stored at 4^{o}C until required for the antibacterial and antifungal bioassays.

The above procedure was repeated for the *Mora gonggrijpii* species used in the current investigation (Fig. 1).

Preliminary Phytochemical Screening

Phytochemical screening of the plant extracts were carried out following a modified method of Cinlei (1957). (See figure 2 & 3).

Reactions carried out in the alcohol extract

Identification of tannins

The alcohol extract (0.5 – 1 ml) was diluted with water (1 – 2 ml) and diluted solution of ferric chloride (light yellow, 2 – 3 drops) added. The occurrence of a blackish blue colour shows the presence of gallic tannins and a green blackish colour indicates catechol tannins.

Identification of alkaloids

The alcohol extract (20 ml) was transferred to a test tube and evaporated on a boiling water-bath. Hydrochloric acid (10 per cent, 5 -10 ml) was added to the residue. Ammonia solution (10%) (pH= 8 - 9) was added to aqueous solution, followed by extraction with ethyl either. The ether was evaporated and the residue was dissolved in hydrochloric acid solution (approximately 1.5 ml, 2 percent). The acidic solution was divided into three test tubes: one as reference and to the other two test tubes 2-3 drops of Mayer's® or Bertrand's® reagents were added. The occurrence of an opalescence or yellowish-white precipitate with Mayer's reagent® or with Bertrand's reagent® indicates the presence of alkaloids.

Reactions carried out in the hydrolysed alcohol extract

To 25 ml alcohol extract hydrochloric acid solution (10 per cent, 15 ml) was added and refluxed for 30 minutes. After cooling, the solution was 3 times extracted using a separating funnel, with ethyl ether (10-12 ml). The ether extracts are

placed together (30-36 ml) and dehydrated with anhydrous sodium sulphate resulting in ether and an aqueous solution.

The ether extract will serve to identify the anthracenosides, coumarins, flavonosides, steroid glycosides, and triterpenes by means of the following series of reactions characteristic of each group:

Identification of anthracenosides [Borntrager's reaction]

The ether extract (4 ml) is concentrated to 2 ml, then ammonia solution (2.5 per cent, 1 -2 ml) is added by shaking. A cherry-red colour of the alkaline solution indicates the presence of emodols (aglycones of anthracenosides).

Identification of coumarin derivatives

The ether extract (5 ml) was evaporated to dryness. The residue was dissolved by heating in 1-2 ml of water. The aqueous solution was divided into two equal volumes, using two test tubes. To one of the tubes, ammonia solution (0.5 ml 10 %) was added; the other tube was used as reference. The occurrence of a blue or green colour indicates the presence of courmins

Identification of steroid glycosides (cardiotonic glycosides or saponins) [Liebermann – Burchand's reaction]

The ether extract (10 ml) was evaporated to dryness. The residue was dissolved in acetic anhydride (0.5 ml) and chloroform (0.5 ml). The solution was transferred to a dry test tube. By means of a pipette concentrated sulphhuric acid (1-2 ml) is added at the bottom. At the interface of the two liquids, a reddish-brown or violet-brown ring with the superior layer being blueish-green or violet indicates the presence of sterols and triterpenes.

Identification of flavonosides (flavones glycosides) [Shibata's reaction]

The ether extract (5 ml) was evaporated to dryness. The residue was dissolved in methanol (50 percent, 1-2 ml) by heating, a piece of magnesium was and 5-6 drops of concentrated hydrochloric acid. The presence of flavonols is indicated by a red colouration of the solution. Flavanones are indicated by an orange coloured solution.

Antimicrobial Assay

Fungal Media [Potato dextrose Agar(PDA)]

200g of potato slices were boiled with distilled water. The potato infusion was used as the water source of media preparation. 25g of dextrose was mixed with potato infusion. 25g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with a sterile 6mm diameter cork borer. The plates with wells were used for the antifungal investigation.

Antifungal activity of the crude plant extract

The following volumes 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 and 1500µL of the crude ethanolic extract were tested against different fungal pathogens for their antifungal activity. The well diffusion and poison plate methods were employed.

Well diffusion method

Antifungal activities of the plant extracts were tested using Well diffusion method (Bauer *et al*., 1996). The prepared culture plates were inoculated with different strains of fungi. Wells were made on the centre of the agar surface with a sterile 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 27±2ºC for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells.

Poison Plate Method

The antifungal activity was determined by the poison food technique (Poonkothai, 2005). The test fungi were allowed to grow on the PDA plate poisoned with known volumes of the crude ethanol extract. The effect of sample on the fungal growth was determined by measuring the diameter of the colony obtained on the poisoned plate.

Streak Plate Method

The prepared culture plates were inoculated with different strains of bacteria using streak plate method (Batista, 1994). The plates were incubated at 37ºC±2ºC for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells.

RESULTS AND DISCUSSION

The crude extracts were prepared, *Talisia squarrosa* formed an orange-brown powder and *Mora gonggrijpii* formed a brown-black powder. In a 100g sample of plant material, *T. squarrosa* and *Mora gonggrijpii* produced 16.9 g (16.9 %) and 21.0g (21.0 %) of crude extract respectively.

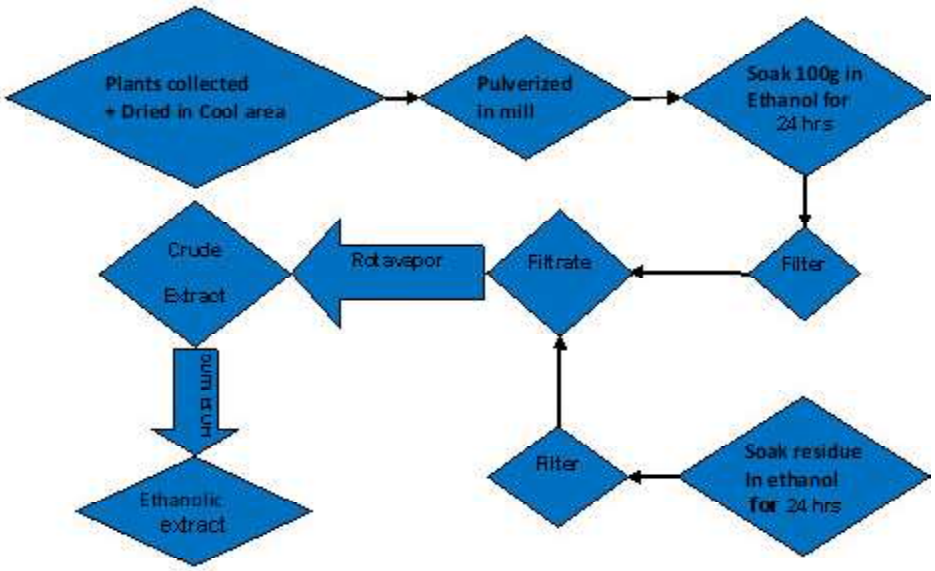

Fig. 1. Flow chart for extraction protocol : Plant collection & extraction

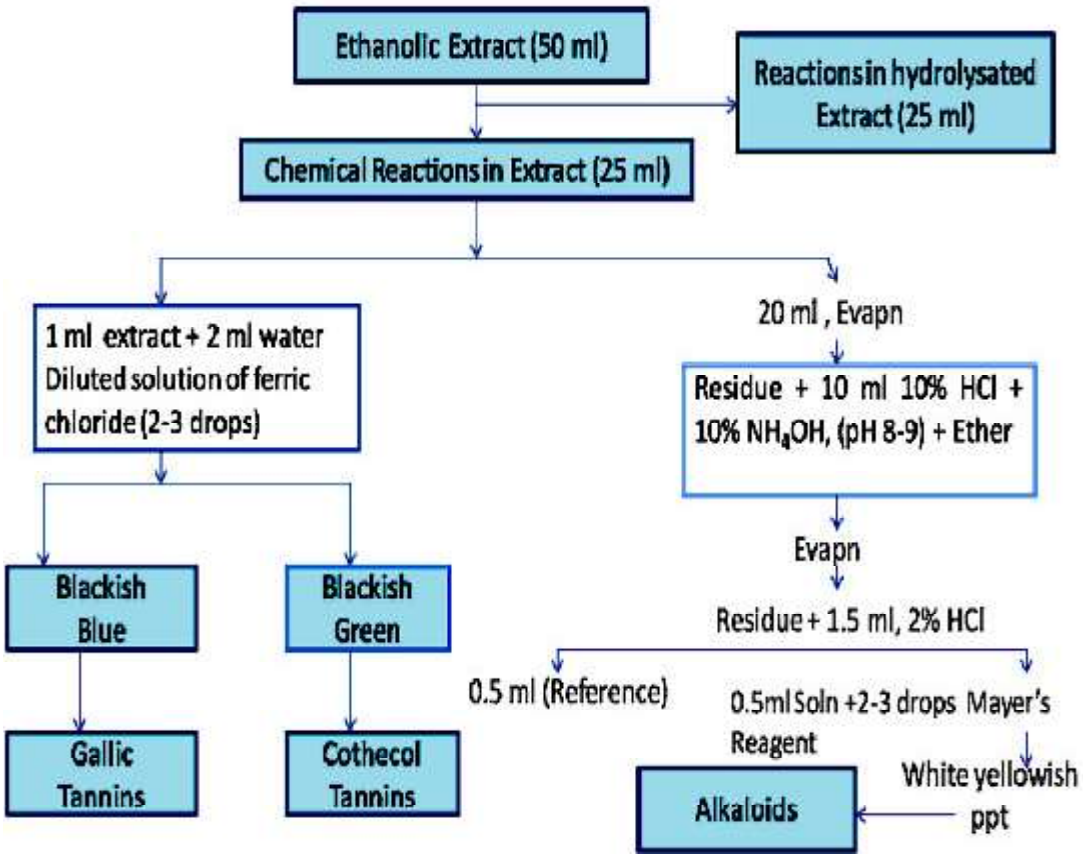

Fig. 2. Flow chart showing the phytochemical screening protcol

Generally, the Sapindaceae and Fabaceae family possess a wide range of biologically active components such as saponins, flavonoids, steroids, proteins and lipids. The preliminary Phytochemical screening of *Talisia squarrosa* and *Mora gonggrijpii* showed the presence of Flavonoids, Glycosides, Tannins, Saponins, anthracenosides and Coumarins. Both plants species lacked the presence of alkaloids (Table1).

The phytochemical examination was done using the crude extracts. The antimicrobial activities observed for plant species in the current investigation, may be due to the presence of the secondary metabolites determined in the phytochemical screening.

Initially, the fungal pathogens were tested using a low concentration range of 100-400µl. At these concentrations no inhibition was observed.

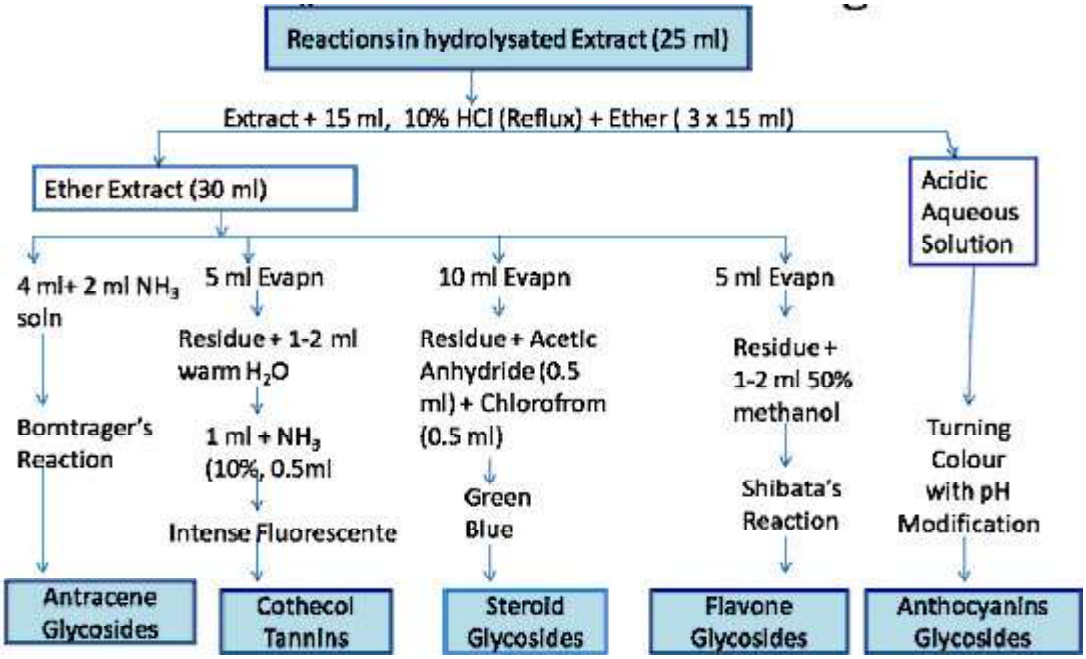

Fig. 3. Flow chart showing the phytochemical screening protcol

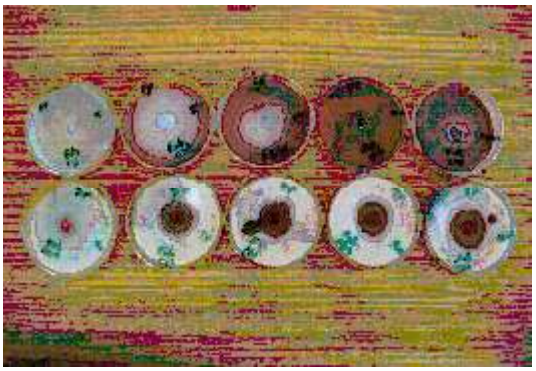

Fig. 4. The effect of extracts (500 - 1000 µl) on test fungi

Fig. 5. The effect of extract (1000 - 1500 µl) on test fungi

Table 1. Phytochemical Screening of Ethanol Extract of *Talisia squarrosa* and *Mora gongrippi*

S. No	Tests	Root of *Talisia* squarrosa	Bark of *Mora* gongrippi
1	Steroids Glycosides	+	+
2	Anthracenoside	+	+
3	Alkaloids	-	-
4	Tannins	+	+
5	Saponins	+	+
6	Flavonoids	+	+
7	Coumarin	+	-

+=positive -=negative

Table 2. Anti-fungal activity of Etanolic Extract of *Mora gongrippi*

		Concentration of ethanolic extract added					
	Volume	1.0 ml	1.1 ml	1.2 ml	1.3 ml	1.4 ml	1.5 ml
Zone of inhibition (cm)	Horizontal	2.5	3.2	2.0	3.7	3.8	4.5
	Vertical	2.0	3.1	2.5	3.7	4.0	4.2

As the concentration increased (from 500 -1000 µl) inhibition became more evident (Fig. 4).

As the concentration was increased from 1ml to 1.5 ml / disc there was further increase in the inhibition zone observed (Fig. 5).

The results of the antimicrobial assay of the ethanolic extract of *Mora gonggrijpii* indicated that the plant exhibited antifungal activity against the tested microorganisms at different concentrations of 1-1.5mL/disc. Zone of inhibition was recorded and presented in (Table 2).

Mora gonggrijpii possesses a number of secondary metabolites which are responsible for the observed antifungal activity. It was reported that *Gliricidia sepium* (Fabaceae) showed antifungal activity. This family generally possesses bioactive compounds.

In the current study *Mora gonggrijpii* extracts demonstrated bioactivity that may explain the significant morphological changes that appear to have occurred in the nature of the fungal growth. They cause mutation of the fungal species. This indicates that the plant species contain one or more compounds which are unfavourable for fungal growth.

The antibacterial assays conducted in this study using the streak plate method revealed that extracts from both *Mora gonggrijpii* and *T. squarrosa* were inactive against the test organisms at the concentrations (100 to 1000 µl). *T. squarrosa* extracts were also inactive against the fungal pathogens used in the current study.

Based on the phtyochemical investigation (Table 1), both plant species possess similar secondary metabolites. Coumarins were present only in the *Talisia squarrosa* extract. The difference in antimicrobial activity may be due to a number of factors such as:

- the absence or presence of coumarin
- the absence or presence of other phytochemicals which were not tested in this study
- the plant species may have different concentrations and proportions of the secondary metabolites.

CONCLUSION

In the present era, plant resources are abundant, but these resources are decreasing rapidly due to the onward march of civilization (Vogel, 1991). It has been widely observed and accepted that the medicinal value of plants lies in

the bioactive phytocomponents present in the plants (Veermuthu *et al*., 2006). In the present study, the active phytocomponents of *Talisia squarrosa and Mora gonggrijpii* were investigated and further the antimicrobial activity of the plant extracts were also tested against plant pathogens from the soil at different concentrations of the extracts to understand the most effective activity. *Mora gonggrijpii* showed maximum zone of inhibition at a concentration of 0.43g/1.5ml against fungal pathogens but was inactive against bacterial pathogens. In the current study Talisia *squarrosa* demonstrated on activity against neither the bacterial strains nor the fungal pathogens used in the study.

It can be concluded from the results obtained in this current study that plants used traditionally may represent new sources of antimicrobials with stable, biologically active secondary metabolites that can establish a scientific base for use in the prevention of pathogenic diseases.

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